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COMMENTARY

The other face of Janus*

Adalberto Laszlo

In this issue, M.H.Hazbón (1) reviews recent advances in molecular methods for early diagnosis of tuberculosis and drug-resistant tuberculosis. This article has much in common with other "state of the art" reviews that have been appearing in the world's literature on molecular tuberculosis (TB) diagnostics for some time now. Most of these reviews are well written, factual accounts of advances in this important field of research. In the last two decades much has been stated and even more has been promised about the role of genomics in providing alternatives to the conventional methods that have been used for over a century.

Emphasis is often given to the development of new diagnostic tools with turn-around times measured in hours rather than days, weeks or months. However, in practical terms, very little of what is often described with such vigorous enthusiasm offers actual relief to the modern TB laboratory specialists or indeed much of a challenge to the old, conventional, smear microscopy and drug susceptibility testing (DST). As the author of the above article laments, only two of these methods, based on nucleic acid amplification and detection of specific mycobacterial sequences and mutations, have been approved (2,3) by the Food and Drug Administration (FDA) in the USA.

Moreover, the approval for the test was given only for AFB smear-positive respiratory specimens in

the case of the Amplicor test, and for either smear positive or negative respiratory specimens in the case of the Enhanced *Mycobacterium tuberculosis* test (E-MTD). This is far from the stellar performance that the sophistication of the technologies used could have allowed us to expect and indeed demand. However, the recent sequencing of *M. tuberculosis*'s genome will eventually lead to the deciphering of the meaning of much genetic information and the creation of truly novel TB diagnostics.

The status of the "replacements" for the other mainstay of TB diagnostics, i.e. drug susceptibility testing, is even less advanced. Not only there has not been regulatory approval for the new tests but it has become evident over time "that the presence or absence of mutations in drug resistance associated genes does not necessarily indicate susceptibility or resistance to the corresponding drug" (1). Isolates with mutations in DR-associated genes can show phenotypic susceptibility and viceversa. The so-called "real resistance-mutations" can be clinically relevant, but their absence doesn't necessarily mean drug susceptibility. These circumstances mean that test results obtained with these methods must always be confirmed by results obtain phenotypic methods (4-6).

The existence of multiple mutations conferring resistance to a given drug have dampened early enthusiasm for these methods. Even DNA sequencing, the "gold standard" for the detection of drug mutations, requires several sequencing reactions per isolate, and is unlikely to be used routinely, except for rifampicin. Other disadvantages include high costs, the need for sophisticated equipment and expertise, the technical difficulty in the performance of this tests and frequent contamination problems. Had some of these tests been developed half a century ago, physicians would have been clamouring for a more

*Janus is the Roman god of gates and doors, beginnings and endings and hence represented with a double-faced head, each looking in opposite directions. It also represents the transition between primitive life and civilization, the countryside and the city and the growing-up of young people.

Correspondencia:
Adalberto Laszlo.
Laszlo@who.int

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"holistic" test, that would also take into account the expression and the modulation of genetic information, in fact a "phenotypic" test!

As for the advantages of these tests, there are obvious and should not be overlooked: some can yield direct, rapid DST results and even detect *M. tuberculosis* at the same time, albeit not always with higher specificity or sensitivity than conventional technology. The molecular analysis of resistance can yield important information not available through conventional testing. Genotypic analysis also can detect specific mutations which identify strains with high levels of resistance or broad spectrum of resistance. As well, the assignment of specific mutations could become an epidemiological marker for resistant strains (7).

So, how have we advanced in the last 20 years in terms of easing the heavy laboratory TB diagnostic burden in high endemic (HEC)-low resource countries? The answer is: not very far, since very few of these tests have reached the level of field testing and none have received regulatory approval in developed countries or are in routine use in NTP's of HEC's.

This brings us to smear microscopy, the mainstay of TB diagnosis in countries bearing 99% of the world's TB disease burden, the old test that the FDA approved tests were designed to replace. Smear microscopy is very "low tech", admittedly not very sensitive and "unpleasant" to perform, it nevertheless yields rapid and specific results in the field. Should we not improve it rather than replace it with a "new" test? Attempts at increasing its sensitivity have unfortunately but predictably failed, i.e. any gain in sensitivity has usually been made at the expense of specificity. There would be, however, much practical value in improving its "ease of use" and making it totally safe for handling clinical specimens on the open bench, at least until new sophisticated research can provide a viable alternative for HEC's. As far as

DST methods are concerned the "gold standard" will remain unchanged for the foreseeable future.

For too long now the bacterial cell has been reduced, in the minds of many, to a small receptacle full of nucleic acids. The reality is by far more complex, genetic information is processed and will be either repressed or expressed. As far as research in applied TB molecular diagnostics is concerned, one would wish that the current fad of genomics could recede somewhat thus giving newer technologies based on alternative disciplines, such as proteomics and lipomics, a much needed chance. Let us hope that the gatekeepers who like the Roman god Janus opened the door into genomics for TB diagnostics, will show their other face and reopen the door leading to other methodological fields of TB diagnostics research.

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